

Composition Studies on Tobacco. XXIX. Strongly Acidic Subfraction of a High Molecular Weight Smoke Pigment

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Introduction

Earlier studies disclosed the presence of a high molecular weight smoke pigment in cigarette smoke condensate, having properties similar to the known pigment of tobacco leaf (7). Structural investigations on the nondialyzable, weakly acidic subfraction of this pigment showed the presence of alkaloids, bases, amino acids, quinic acid, iron and a silicone (4). Reexamination of a subfraction of the leaf pigment, previously shown to contain sugars, polyphenols, amino acids and iron, also demonstrated the presence of alkaloids, bases and a silicone therein (3). Differences were observed in the molecular weights and in the proportions of alkaloids and volatile bases in the two pigments and on this basis it was postulated that the smoke pigment originates from the leaf pigment during burning. To obtain further information on the smoke pigment, a detailed study has now been made of the nondialyzable strongly acidic subfraction of the pigment.

Methods

The isolation of the crude, strongly acidic subfraction of the smoke pigment was described previously (4). This subfraction comprised about 1.0% of the weight of smoke condensate and was extracted continuously with ether for 16 hrs. in a manner similar to the weakly acidic subfraction. The ether-insoluble material was dialyzed against 0.01 M

phosphate buffer (pH 10.0) for about 150 hrs. with daily changes of buffer (total, 200 l buffer/14.6 g pigment). The dialyzable and nondialyzable materials were independently precipitated by acidification with conc. hydrochloric acid to pH 1.0.

Acidic hydrolyses, alkaline fusions and molecular weight approximations were performed as previously described (4). Amino acids were determined on an automatic amino acid analyzer, using a published procedure (11). Quinic acid, alkaloids, and bases were analyzed by the methods outlined earlier (4,6). The ether solution of neutrals and acids remaining after removal of the bases from the alkaline fusion products was extracted with aqueous alkali which removed weak and strong acids. The aqueous layer was acidified to pH 6.0 and extracted continuously with ether for 16 hrs., remov-

ing the weak acids. The aqueous solution was then adjusted to pH 1.0 and again extracted continuously with ether, removing the strong acids. The two ether solutions were dried and concentrated independently to 1.0 ml. Total phenols were determined on the concentrate of weak acids by the method of Lorentzen and Neurath (5). Individual phenols and acids in the concentrates were isolated and identified by a previously described gas chromatographic method using a diethylene glycol adipate polyester— H_3PO_4 column operated at 175°C (7).

Results and Discussion

Table 1 shows representative differences in yields of the weakly and strongly acidic subfractions of the smoke pigment (SP). Of the two fractions, the strongly acidic frac-

Table 1. Yields of strongly and weakly acidic subfractions of smoke pigment

Fraction	Yield*	
	Weakly acidic	Strongly acidic
Total	4.80	1.51
Ether-soluble	1.44	0.04
Ether-insoluble	3.36	1.47
Ether-insoluble, nondialyzable	1.68	.34
Ether-insoluble, dialyzable**	1.68	1.13

*Percentage of original smoke condensate.

**By difference

tion contains proportionally less ether-soluble material and more dialyzable substance. The ether-insoluble dialyzable strongly acidic subfraction is not homogenous since selected properties of the materials dialyzed at 24 and 48 hrs. show distinct differences (Table 2). Apparently, some variations occur in the molecular weights and elemental analytical values of subfractions from the strongly acid pigment (SA) obtained from different batches of condensate: in a previous isolation (4), sulfur was absent in SA prior to ether extraction and dialysis, and the range of approximate molecular weights was 20,000 to $\geq 100,000$ in the subfractions. The influence of condensate age in these variations is unknown. However, pigments from freshly prepared smoke condensates of the four major cigarette types have distributions of molecular weights which are similar to those of smoke condensates of blended cigarettes aged for several months (1). The acidic pigment is also obtained when smoke is collected in aqueous media rather than cold traps.

Acidic hydrolysis of the nondialyzable strongly acidic subfraction (NSA) gave about 20 substances which reacted with ninhydrin including nineteen amino acids which were generally similar to those obtained in the nondialyzable weakly acidic subfraction (NWA) (4). The major amino acids in NSA were glycine, alanine, 1-methylhistidine, glutamic acid and valine. The amount of hydrolyzable ninhydrin-positive substances in NSA (about 3.5%) was much higher than that obtained with NWA (about 0.9%). Also, the level of quinic acid released by acidic hydrolysis was higher in NSA (about 0.12%) compared to NWA (<0.04%).

The following alkaloids and bases were found in alkaline fusion products from NSA: 3-pyrroline, pyridine, β - and α -picolines, a mixture of collidines, 3-vinylpyridine, pyrrole, nicotine, nornicotine and metan nicotine. Quantitatively, the picolines and collidines were the major components, constituting about 80% of total bases, and the largest single component was β -picoline, amounting to about 40%. The total level of bases and alkaloids released by alkaline fusion was about 0.5% of NSA. NWA contains less total alkaloids and bases (about 0.3%) and different proportions of these components.⁽³⁾

An intensive study was made of the volatile acidic and phenolic products from the alkaline fusion

Table 2. Selected properties of dialyzable and nondialyzable strongly acid subfractions of smoke pigment

Analysis*	Dialyzable at		Nondialyzable
	24 hrs.	48 hrs.	
C	61.67	65.15	63.54
H	5.46	5.18	5.09
N	4.75	5.17	5.65
S	1.15	1.14	1.09
Ash	3.75	0.65	3.26
Equivalent weight	520	490	380
Melting point	>320° (d.)	>320° (d.)	>320° (d.)
Molecular weight	4000	7000	15,000-100,000

*Elements and ash are expressed as percentage of sample

of NSA. Table 3 gives the identified components and quantities thereof obtained from these fractions. The values include amounts of acids found in both weakly and strongly acidic fractions of the alkaline fusion products. On DEGA columns, phenol and *o*-cresol are unresolved and *m*- and *p*-cresol are also inseparable; however, the fusion products contained no *o*- or *m*-cresol in these peaks based on the infrared spectra. The total phenols amounted to about 6.6% of NSA, and phenol and *p*-cresol comprised about 14% of the total phenols. The remainder may be phenolic fusion products of quinic or chlorogenic acid, e.g., protocatechuic acid or other unidentified products. The levels of total chlorogenic acid (hydrolyzable plus nonhydrolyzable) in the unfractionated smoke pigment is known to be about 6.0%, (8) of which <1.0% is hydrolyzable polyphenol.

These phenols and acids may be present as moieties in the pigment or may be formed as degradation products of amino acids, (9) quinic acid or unknown components of the pigment during alkaline fusion. Many of the amino acids in the hydrolysates contain carbon skeletons equivalent in chain length and configuration to the fatty acids isolated. Acetic and formic, which are formed in relatively high concentrations, may arise from alkaline fusion of a variety of moieties, including sugars, chlorophyll and related substances (10) some of which may be present in unhydrolyzable linkages and have not been identified as components of the pigment. In general, the action of hot, molten alkali on many groups of organic compounds has been largely uninvestigated. The isolation of these acids in levels higher than the amino acids does not eliminate the latter as a possible source since large amounts of unhydrolyzable amino acids may be linked in the pigment. The pres-

ence of octanoic acid may indicate that an *n*-C₈ moiety other than an amino acid is present in the pigment structure since no common amino acid with this skeleton is known. Nicotine, (3) nornicotine, anabasine and myosmine (2) are stable to alkaline fusion and should not have contributed significantly to the isolated acids.

Because of the relatively small amount of available material, determinations of iron and silicon in the ash and of the presence of silicones in the alkaline fusion products could not be made.

Summary

The strongly acidic subfraction of a high molecular weight pigment from smoke condensate was shown to

Table 3. Volatile acids and phenols in alkaline fusion products of nondialyzable, strongly acidic subfraction

Component	Percentage of pigment
Acids	
Acetic and formic acids	13.4
Propionic	3.6
Isobutyric	0.2
Butyric	0.6
Isovaleric	0.9
Valeric	0.1
Isocaproic	0.4
Caproic	0.1
Heptanoic	~0.1
Octanoic	~0.1
α -Hydroxyisovaleric	0.4
Phenols	
Phenol	0.1
<i>p</i> -Cresol	0.8
Total	20.8

be qualitatively similar but quantitatively different than the weakly acidic subfraction. Both subfractions contain amino acids, quinic acid, alkaloids and bases but the ether-insoluble, nondialyzable, strongly acidic subfraction (NSA) contains larger amounts of such substances released by acidic hydrolysis or alkaline fusion compared to the comparable weakly acidic subfraction. Significant amounts of volatile phenols and acids are released from NSA by alkaline fusion and the possible origin of these components is discussed.

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